REMARKS

Status

Claims 87-129, which were presented by a preliminary amendment, were at issue in connection with the present Office Action. This response does not cancel any claims, and adds new claims 130-140. Accordingly, it is claims 87-140 which are at issue in this response.

The Office Action

In the Office Action mailed September 13, 2007, claims 87-129 were rejected. Specifically, claims 88 and 90 were objected to as reciting improper Markush language. Claims 88-89, 91, 96-98, 102, 104-106, 111-114, 116, 118, 122, 123 and 125-127 were rejected under 35 U.S.C. §112, second paragraph, for particular noted informalities.

Claims 87-89, 97-100, 107, 109, 112, 117-118, 120-123 and 129 were rejected under 35 U.S.C. §102 as being anticipated by U.S. Patent 5,428,541 of Lea.

Claims 87, 90, 95-96, 103, 113-114 and 116 were rejected under 35 U.S.C. §103 as being unpatentable over the Lea '541 patent taken in view of the published patent application of Fan 2002/0001801.

Claims 87-88, 91, 102 and 107 were rejected under 35 U.S.C. §103 over Lea '541 taken in view of U.S. Patent 5,547,849 of Baer.

Claims 87-88, 92-93, 103 and 110-111 were rejected under 35 U.S.C. §103 as being unpatentable over Lea '541 taken in view of U.S. Patent 5,728,527 of Singer.

Claims 87-88, 92 and 94 were rejected under 35 U.S.C. §103 as being unpatentable over Lea '541 taken in view of U.S. Patent 5,726,009 of Connors.

Claims 87, 101, 108 and 110 were rejected under 35 U.S.C. §103 as being unpatentable over Lea '541 taken in view of U.S. Patent 5,573,909 of Singer.

Claims 87, 104 and 107 were rejected under 35 U.S.C. §103 over Lea '541 taken in view of U.S. Patent 5,849,508 of Brechot.

Claims 87, 105 and 107 were rejected under 35 U.S.C. §103 as being unpatentable over Lea '541 taken in view of U.S. Patent 5,691,147 of Draetta.

Claims 87, 106 and 107 were rejected under 35 U.S.C. §103 as being unpatentable over Lea '541 taken in view of U.S. Patent 6,379,882 of Bitler.

Claims 87, 115 and 128 were rejected under 35 U.S.C. §103 as being unpatentable over Lea '541 alone.

Claims 87 and 119 were rejected under 35 U.S.C. §103 as being unpatentable over Lea '541 taken in view of U.S. Patent 6,100,535 of Mathies.

Claims 87 and 124 were rejected under 35 U.S.C. §103 as being unpatentable over Lea '541 taken in view of U.S. Patent 5,115,304 of Yoshikawa.

Claims 87 and 125-127 were rejected under 35 U.S.C. §103 as being unpatentable over Lea '541 taken in view of U.S. Patent 5,850,485 of Hart.

Applicant thanks the Examiner for the Office Action, for the search, for the suggestions regarding correction of particular informalities in the claims, and for the thorough explanation of the basis of the rejections.

The Present Invention

Applicant will briefly recapitulate the principles of the present invention, as now claimed, so as to better differentiate this invention from the prior art. The present invention is directed to a method for the detection and analysis of particles in a liquid material through the use of a reagent which comprises a labeled targeting species. The targeting species is capable of selectively binding to analyte detectable positions on the particle and the labeling agent provides

an electromagnetic signal such as a fluorescent signal. The method of the present invention is directed to those situations wherein the number of analyte detectable positions on the particles is very low (less than 1×10^6 positions per particle). As a consequence, the detectable signal produced by the reagent material will be quite low. This is in contrast to prior art systems such as those which rely upon fluorescent labeling of DNA, which is present in abundant amounts in cellular material so that typical particles will have 3×10^9 detectable positions per particle; and, hence, in prior art processes, a large electromagnetic signal can be captured from each cell in a short period of time. Accordingly, in such prior art methods, the analysis may be made on a moving sample which flows past a detection element. In contrast, the signal produced by particles being analyzed in the present invention is at least 1,000 times lower, and hence too weak to be measured in a flow system. The present invention relates to the finding that it is possible to detect particles being labeled at only a few positions by using a sample which is at rest in a system with relatively low levels of optical signal magnification (typically ranging from unity to no more than 20:1).

The Rejection under 35 U.S.C. §102

Claims 87-89, 97-100, 107, 109, 112, 117-118, 120-123 and 129 were rejected under 35 U.S.C. §102 as being anticipated by the Lea '541 patent. The method according to the Lea '541 patent relates to a flow measurement system where a fluorescent, optical signal, generated by irradiation of particles having a labelled reagent bound thereto, produces a high intensity optical signal which is measured as the sample flows through a sample compartment. As discussed above, the present invention is directed to systems in which an electromagnetic signal generated by particles being analyzed is very faint. The present invention recognizes that such signals may be effectively measured in a method wherein the sample is at a standstill during

measurement and wherein low levels of optical magnification are employed. Independent claim 87 has been amended to emphasize and point out these differences. In that regard, the first paragraph of the claim has been amended to clarify that the number of detectable positions **per particle** is less than 1×10^6. A similar amendment is made to the second paragraph. Claim 87 has furthermore been amended by deleting the phrase "wherein the representation is subject to a linear enlargement" in lines 25-26. This deletion does not expand the claims beyond their original scope or beyond the scope of the disclosure as filed and makes clear that the low level of optical magnification may include a ratio of 1:1 (no magnification) as well as magnifications ranging up to 20:1. This amendment has been made to meet the Examiner's comment under item 4 relating to reference made to claim 87, in claim 122.

In line 24 of claim 87, the phrase "the sample" has been replaced by the phrase "the mixture" to clarify that the electromagnetic signals are emitted from the mixture of the liquid material and the reagent material in a sample compartment. Lines 30-31 have been amended to correct an obvious typographical error and now read "so that the ratio of a linear dimension of the image". Claim 87 has furthermore been amended by introducing the limitation that the sample is at a standstill during the optical detection. Basis for this amendment can be found on page 25, lines 1-9 of the application as filed.

In view of the present amendment, the rejection under 35 U.S.C. §102 over Lea '541 is overcome. The Lea patent does not show any system wherein measurements are carried out on particles having low levels of detectable positions wherein that measurement is carried out on a non-flowing (standstill) material. Lea is clearly directed to high speed flow systems utilizing particles generating intense signals. As such, the rejection is overcome.

Applicant further respectfully suggests that there is no teaching in Lea which would suggest modifying the disclosed method to carry out analysis on non-flowing samples. All teaching in Lea is to flow systems. Furthermore, Lea is analyzing materials which generate a high intensity signal and hence does not recognize the problem addressed by the present invention. Therefore, all teaching in Lea is directed to flow systems and away from standstill systems of the present invention.

The Rejections under 35 U.S.C. §103

Various of the claims have been rejected under 35 U.S.C. §103 over the Lea base reference taken in combination with various other prior art references as discussed above. Given the general inapplicability of the Lea reference to the present invention, and further in view of the explicit teaching away in Lea of the use of a non-flow system for measuring fluorescently tagged particles, further rejections under 35 U.S.C. §103 are inappropriate. Applicant will further discuss the general inapplicability of each of the specific rejections based upon Lea hereinbelow.

A. The invention is non-obvious in view of Lea et al. (US 5,428,451)

The method according to Lea et al. relates to a flow system. It can hence only be used for assessing quantitative and qualitative parameters of particles with a large amount of analyte detectable positions such as when acridine orange (col. 5, line 35) is used to stain the DNA of cells. It is described how the statistical quality relating to the individual particles can be improved by combining information from several images, where the particle have moved between the individual images (col. 5, lines 54-60). But when none of these images captures a sufficiently strong signal from a given particle to provide a sufficiently high signal to noise ratio in each image, the electromagnetic signals from the individual images cannot be combined in a

reliable manner. The method according to Lea et al. is hence unsuitable for assessing particles with a low number of analyte detectable positions.

The person skilled in the art would hence not have a reasonable expectation of success if he or she should consider attempting to make an assessment of quantitative and qualitative parameters of particles with a low amount of analyte detectable positions using the method according to Lea et al.

It is mentioned that biological cells can be counted, but no hint is provided to consider using the method of Lea et al. in the special case of assessing quantitative or qualitative parameters of particles, on which labelling agents bind to analytes present only in a low number on each particle.

Claim 87 and all claims dependent thereon are hence non-obvious in view of Lea et al.

B. The invention is non-obvious in view of Lea et al. and Fan et al. (US 2002/0001801)

Fan et al. discloses a method for immobilizing analytes from a patient sample on microspheres/beads. The microspheres only act as mediators for the assessment of the analytes from the sample in contrast to the situation in the method according to the present invention, wherein the particle is the natural host of the analytes. There is hence a clear difference between the microspheres of Fan et al. and the particles of the present invention. The nature of the microspheres/particles of Fan et al. is given in paragraph [0052]. The microspheres comprising the analytes are immobilized at discrete and well defined sites in an array defined on solid supports as described in paragraphs [0034] to [0050]. The correct assessment of the analytes is linked to the precise location of the microspheres at these sites (lines 14-16 of paragraph [0022]). The location can for instance be defined by selectively etching the cores of a fiber optic bundle (paragraphs [0043] and [0048]). In contrast, Lea et al. captures signals from the same particle at

several different positions along the flow direction and subsequently combines these signals to provide an image of the stained particles in the sample (Lea et al., col. 5, lines 54-62). The array based method according to Fan et al. is hence fundamentally different from the flow system of the method according to Lea et al.

The mere fact that Fan et al. describes that it is possible to determine the content of particles in an array, wherein each particle is located at a specific site, does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles based on labelled analytes having less than 1×10^6 positions in a completely different system. The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Furthermore, it is simply not possible to construct the method according to the present invention by arbitrarily choosing from the combined teachings of Lea et al. and Fan et al. without the use of impermissible hindsight.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Fan et al.

C. The invention is non-obvious in view of Lea et al. and Baer et al. (US 5,547,849)

In the method according to Baer et al., a highly focused laser beam is scanned over a capillary tube constituting the sample compartment. The combined effect of the high intensity of the **highly focused laser** beam and the option of using relatively long exposure times, allows for an assessment of qualitative and quantitative parameters of particles with a low number of detectable positions. In Lea et al., the sample flows through the sample compartment. When the

sample is flowing during the optical inspection, an image of the entire inspected section of the sample compartment must be taken instantaneously in order to probe the entire sample volume. If a highly focused laser beam is scanned over the sample compartment while the sample is flowing, there will unavoidably be sections of the sample that are not exposed to the laser beam and the optical inspection will provide an incomplete detection of the particles in the sample. The method according to Lea et al. is hence incompatible with the method according to Baer et al. Furthermore, Baer et al. does not disclose a method wherein the lenses positioned between the sample compartment and the detection device provide a magnification below 20:1.

The shortcomings of Lea et al. with respect to the assessment of quantitative and qualitative parameters of particles with less 10^6 analyte detectable positions particles cannot be corrected by modifying the method using features of a system for scanning a highly focused laser beam over the sample instead of acquiring an image of the sample in the sample compartment. Since the two systems cannot be combined in a reasonable manner, a person skilled in the art of detecting particles using a flow system would not be prompted to search in Baer et al. for hints to the assessment of qualitative or quantitative parameters of particles that inherently cannot be detected when using the flow system according to Lea et al.

The mere fact that Baer et al. describes that it is possible to determine the content of particles by using a scanning laser does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles based on labelled analytes having less than 1×10^6 positions in a completely different system. The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Furthermore, it is simply not possible to construct the method according to the present invention by arbitrarily choosing from the combined teachings of Lea et al. and Baer et al. without the use of impermissible hindsight.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Baer et al.

D. The invention is non-obvious in view of Lea et al. and Singer et al. (US 5,728,527)

In Singer et al. the assessment of the species that is detected is based on taking photomicrographs (col. 10, lines 22-29) of stained cells fixated on e.g. a glass slide (col. 9, line 65 – col. 10 line 3 and in col. 12, line 60 – col. 13, line 2). The cells that are to be analyzed are actually grown on glass cover slips (col. 11, lines 33-39). Lea et al. on the other hand relates to a method for the detection of particles in a liquid sample and hence the detection methods of Lea et al. and Singer et al. cannot be combined. A person skilled in the art of flow systems such as the one disclosed in Lea et al. would hence not be prompted to combine the teachings of Lea et al. and Singer et al. as these two methods relates to incompatible detection methods.

The mere fact that Singer et al. describes that it is possible to determine the content of particles fixated on a glass slide does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles in a liquid sample based on labelled analytes having less than 1×10^6 positions in a completely different system. The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Furthermore, it is simply not possible to construct the method according to the present invention by arbitrarily choosing from the combined teachings of Lea et al. and Singer et al. without the use of impermissible hindsight.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Singer et al.

E. The invention is non-obvious in view of Lea et al. and Connors et al. (US 5,726,009)

Connors et al. disclose a method for determining viability or proliferation of certain cells in a tissue sample. The viability of the cells can be tested by exposing them to certain metabolic markers (col. 5, lines 54-63) or by detecting the changes in a metabolic substrate that is converted when reacting with the viable cells (col. 6, lines 45-51). The optical characterization is performed using a microscope setup (col. 7, lines 35-37). As described in col. 3, lines 45-67 the three-dimensional structure of the tissue sample is maintained during culturing and subsequent optical characterization. The method according to Connors et al. is hence fundamentally different from the method according to Lea et al. wherein a liquid sample flows by the detection element. The teachings of Lea et al. and Connors et al. can hence not be combined in a reasonable manner.

In relation to the magnification used in the optical setup, Connors et al. describes in col. 7, lines 35-37 that a magnification of 200:1 is used. There is hence no disclosed in Connors et al. of a method wherein a magnification below 20:1 is used.

Connors et al. does not provide any hints to realizing the viability testing on a liquid sample, wherein the particles/cells that are to be assessed are dispersed in the liquid. The mere fact that Connors et al. describes that it is possible to determine the content of particles in a tissue sample while maintaining the structure of the tissue does not provide the skilled person with any

expectation of success with respect to the possibility of assessing particles in a liquid sample based on labelled analytes having less than 1×10^6 positions in a completely different system. The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Furthermore, it is simply not possible to construct the method according to the present invention by arbitrarily choosing from the combined teachings of Lea et al. and Connors et al. without the use of impermissible hindsight.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Connors et al.

F. The invention is non-obvious in view of Lea et al. and Singer et al. (US 5,573,909)

Singer et al. describes in US 5,573,909 a method for labelling target materials using surface coated fluorescent microparticles. When addressing the optical detection of the labelled target materials (col. 20, line 33 – col. 21, line 30), Singer et al. provides only methods, wherein the labelled target material is immobilized on a solid or semi-solid support. This is also illustrated in the Examples, where the labelled target material is spotted onto nitrocellulose filters (Examples 9, 10, 12 and 17), fixed on microscope slides and/or cover slips (Examples 11, 15, 16, 19 and 20), or transferred to a membrane for Western blotting (Examples 14 and 18). There is hence a significant and crucial difference between the detection technique described by Lea et al. where the liquid sample is flowing during the optical inspection and the detection technique according to Singer et al. The teachings of these two references can hence not be combined.

The mere fact that Singer et al. describes that it is possible to determine the content of particles that are immobilized on a solid support does not provide the skilled person with any

expectation of success with respect to the possibility of assessing particles in a liquid sample based on labelled analytes having less than 1×10^6 positions in a system wherein the particles are dispersed in a flowing liquid. The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Furthermore, it is simply not possible to construct the method according to the present invention by arbitrarily choosing from the combined teachings of Lea et al. and Singer et al. without the use of impermissible hindsight.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Singer et al. (US 5,573,909).

G. The invention is non-obvious in view of Lea et al. and Brechot et al. (US 5,849,508)

Brechot et al. presents a process for the detection of cell proliferation. The detection is based on techniques such as Northern blotting (Fig 1C, col. 5, line 25), Southern blotting (col. 3, line 48), Western blotting (col. 5, line 25 and col. 6, lines 41-43) and ELISA (col. 6, lines 32-34). All of these methods are based on immobilizing the labelled target material to a solid support and hence have no common ground with the flow based system according to Lea at al. The teachings of these Brechot et al. and Lea et al. can hence not be combined.

The mere fact that Brechot et al. describes that it is possible to determine the content of particles that are immobilized on a solid support does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles in a liquid sample based on labelled analytes having less than 1×10^6 positions in a completely different system. The Examiner tends to believe that if a given particle labelled at given analytes is detectable in

one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Furthermore, it is simply not possible to construct the method according to the present invention by arbitrarily choosing from the combined teachings of Lea et al. and Brechot et al. without the use of impermissible hindsight.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Brechot et al. (US 5,849,508).

H. The invention is non-obvious in view of Lea et al. and Draetta et al. (US 5,691,147)

Draetta et al. relates to the discovery of novel proteins capable of binding the human cycline dependent kinase 4 (CDK4). In col. 25, line 67 to col. 26, line 35 is briefly described the detection of lesions in genes encoding a protein binding to the CDK4. It is mentioned that the CDK4 binding protein can be detected in an immunoassay (col. 26, lines 34-35). But Draetta et al. provides no specific information relating to the method for the optical detection of these species. Since there is no disclosure in Lea et al. of a method for the assessment of particles with a low amount of analyte detectable positions while the sample is at standstill, the combination of Lea et al. and Draetta et al. does not provide any hints to making an assessment of such particles using the method disclosed in the amended claims of the current invention.

The mere fact that Draetta et al. describes that it is possible to determine the content of particles using for instance an immunoassay does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles in a liquid sample based on labelled analytes having less than 1×10^6 positions with the method according to the amended claims of the current application. The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the

same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Draetta et al. (US 5,691,147).

I. The invention is non-obvious in view of Lea et al. and Bitler et al. (US 6,379,882)

Bitler et al. describes a method for selecting therapeutic agents for in vivo treatment. A number of different methods for detecting fluorescently stained cells are briefly mentioned: flow-cytometry (col. 12, line 48), microscopy (e.g. col. 13, lines 3-4; col. 13, lines 9-12; and col. 14, line 5) and so forth. Similar to the method according to Lea et al., the flow-cytometer technique is known to the person skilled in the art as being relevant for the assessment of particles that are stained with a large number of fluorescent molecules. Microscope based methods on the other hand have proven capable of assessing particles with a low amount of analyte detectable positions. Bitler et al. provides no hints to using the flow based technique of Lea et al. for assessing particles that a person skilled in the art of detecting cells would assess by using for instance microscope based techniques. Furthermore, Bitler et al. provides no hints to adapting the method according to Lea at al. to having the liquid sample at standstill during the optical detection.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Bitler et al. (US 6,379,882).

J. The invention is non-obvious in view of Lea et al. and Mathies et al. (US 6,100,535)

Mathies et al. describes a system for detecting electrophoretic separations in capillary tubes. When using a system for electrophoretic separation to analyse samples containing variously-sized particles (such as stained DNA fragments), the particles are spatially separated

by an electrical field applied over the sample. When subjecting a sample containing a number of differently-sized DNA fragments stained with a fluorescent dye to an electrophoretic separation, the DNA fragments of same size will form a coherent population at a location in the capillary tube determined by the fragment size. Fluorescence emitted from the stain molecules when exposing the capillary tubes to an excitation light source is seen as bands. The content of the examined sample is identified by comparing the position of the bands of the sample under analysis with the location of the bands from stained DNA fragments in a reference sample. In the method according to Lea et al., electromagnetic signals from the individual particles are identified as distinct from the background and the liquid sample flows during the optical inspection. The system according to Mathies et al. hence relates to a measurement technique that is incompatible with the method according to Lea et al. and the person skilled in the art would not be prompted by Mathies to adapt the system according to Lea et al. to be similar to the system according to the present invention.

The mere fact that Mathies et al. describes that it is possible to separate DNA fragments in a sample and detect populations of these fragments from their locations, does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles based on labelled analytes having less than 1×10^6 positions in a completely different system. The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Furthermore, it is simply not possible to construct the method according to the present invention by arbitrarily choosing from the combined teachings of Lea et al. and Mathies et al. without the use of impermissible hindsight.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Mathies et al.

K. The invention is non-obvious in view of Lea et al. and Yoshikawa et al. (US 5,115,304)

We traverse the Examiner's statement that it would have been obvious for a person skilled in the art of detecting particles in a liquid flow system to modify the system according to Lea et al. with the teachings relating to a photocopying system provided by Yoshikawa et al. Furthermore, Yoshikawa et al. provides no hint to the detection of particles with a low amount of analyte detectable positions while the liquid sample is at standstill.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Yoshikawa et al. (US 5,115,304).

L. The invention is non-obvious in view of Lea et al. and Hart (US 5,850,485)

The system according to Hart relates to Particle Image Velocimetry analysis of flow characteristics. A method for image correlation is described and it is mentioned that individual particles can be recorded. But Hart provides no hint to the detection of particles with a low amount of analyte detectable positions while the liquid sample is at standstill.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Hart (US 5,850,485).

The Rejections under 35 U.S.C. §112

By the present amendment, Applicant has addressed all of the informalities noted by the Examiner, and Applicant again thanks the Examiner for these helpful comments. In view of these amendments, all objections and rejections under 35 U.S.C. §112 are overcome.

Conclusion

In view of the amendments and remarks presented herein, Applicant respectfully submits that all objections and rejections are overcome. The application is in condition for allowance. Should the Examiner have any questions, comments or suggestions which would place the application in still better condition for allowance, they should be directed to the undersigned attorney.

Dated:

Respectfully submitted,

By_____

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